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CLINICAL STUDIES

Platelet Prostacyclin Binding in Coronary Artery Disease

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Reduced responsiveness of platelets to prostacyclin, reported in vitro in patients with coronary artery disease, has been thought to be a factor predisposing toward coronary thrombosis and vasospasm as a result of enhanced in vivo release of cyclic endoperoxides and thromboxane A_2 by the platelets. In this study, specific binding of prostacyclin to intact platelets was determined in patients with coronary artery disease by direct binding studies using 9-^{3}H-prostacyclin sodium salt. In addition, the inhibitory effect of prostacyclin on primary aggregation induced by adenosine diphosphate and cyclic adenosine monophosphate (cyclic AMP) accumulation stimulated by prostacyclin was examined. Twenty patients with angiographically documented coronary artery disease and stable angina, 8 patients with acute myocardial infarction, 14 healthy volunteers and 10 patients with normal angiograms were studied.

In patients with stable angina, binding capacity and affinity of platelet prostacyclin binding sites and prostacyclin-induced cyclic AMP accumulation were not different from those of control subjects. In patients with acute myocardial infarction, however, binding capacity of platelet prostacyclin receptors was significantly reduced (0.69 ± 0.45 versus 1.35 ± 0.37 pmol/10^9 platelets, $p = 0.001$) and the postreceptor response, represented by platelet responsiveness to prostacyclin and prostacyclin-induced cyclic AMP synthesis, was impaired.

Because all patients with myocardial infarction were receiving intravenous heparin and nitroglycerin, which might interfere with platelet prostacyclin binding, competition experiments were performed in vitro. Neither heparin (3 to 250 IU/ml) nor nitroglycerin (0.8 to 22 μM) displaced specifically bound 9-^{3}H-prostacyclin. L-Epinephrine in concentrations up to 10 μM also exhibited no competition with specific platelet prostacyclin binding.

Thus, the data provide no evidence that reduction of platelet prostacyclin receptor density or prostacyclin-induced cyclic AMP accumulation is an additional pathogenetic mechanism predisposing to thrombotic occlusion or vasospasm in stable angina. The reduction of platelet prostacyclin binding observed in acute myocardial infarction, however, might induce further intracoronary thrombotic events or a decrease in coronary blood flow due to vasospasm, particularly in combination with locally reduced prostacyclin synthesis in atheromatous plaques.

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There is considerable evidence (1,2) to support the concept that platelet activation plays an important role in the pathogenesis of cardiovascular disorders. After pacing-induced angina (3,4) and in patients with angina at rest (5), increased concentrations of thromboxane B_2 have been found in coronary sinus blood and have been interpreted to indicate platelet activation in the coronary vasculature during ischemia. As shown by angiographic investigation (6,7), coronary thrombosis or vasospasm (or both) is a predominant finding in the early hours of myocardial infarction. Arachidonate metabolites produced by the platelet cyclooxygenase pathway are known for their vasospastic, proaggregatory and arrhythmogenic properties (8–10). On the other hand, efficient vasodilation and inhibition of platelet function can be achieved by prostacyclin, an unstable arachidonate metabolite produced by the endothelium (11). The relative concentrations of prostacyclin and thromboxane A_2 at the site of thrombus formation have been postulated to be essential determinants regulating hemostatic balance and vascular tone (12).

Enhanced susceptibility of platelets to proaggregating stimuli with the concomitant increase of eicosanoid formation might explain the increased thromboxane formation in the coronary vasculature during ischemia (13–15). However, impaired release of prostacyclin by damaged endo-

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thelium (16) and, in particular, reduced responsiveness of platelets to prostacyclin (17,18) might favor additionally the in vivo synthesis of platelet-derived endoperoxides and thromboxane A_2. Specific platelet prostacyclin receptors have been shown to exist (19,20). In platelets, ligand-receptor coupling is known to cause activation of platelet adenylate cyclase (21). The resulting increase of intracellular cyclic adenosine monophosphate (cyclic AMP) inhibits platelet phospholipases by regulation of free cytosolic calcium and controls the synthesis of cyclic endoperoxides (PGG_2, PGH_2) and thromboxane (22,23). Thus, binding of prostacyclin to platelets seems to have a key role in the modulation of local interactions between platelets and the endothelium.

The present study was performed to compare specific platelet 9-^{3}H-prostacyclin binding (affinity and number of binding sites) in patients with acute myocardial infarction, patients with stable angina, angiographically normal patients and healthy volunteers. Furthermore, the postreceptor responses, that is, prostacyclin-induced cyclic AMP accumulation and antiaggregatory effectiveness of prostacyclin on adenosine diphosphate-induced platelet aggregation, were determined.

Methods

Subjects. The study subjects consisted of four subgroups: *Group I comprised 14 healthy young men, all nonsmokers,* with no family or personal history of peripheral vascular or coronary artery disease (24). None had hypertension or elevated plasma lipids, or was taking long-term maintenance or intermittent medication or any drug known to interfere with platelet function.

Group II comprised 20 patients hospitalized for coronary angiography. All had a history of typical exertional chest pain diagnosed as angina pectoris. None had diabetes or a history of peripheral vascular disease. Ten patients had a previous myocardial infarction with residual electrocardiographic changes (Q waves). Coronary angiography was performed using the Judkins technique. The angiograms were reviewed by at least two experienced cardiologists who were unaware of the biochemical data. The extent of the stenoses was graded and the angiographic severity of coronary artery disease was calculated using the Gensini score (25). All 20 patients were taking oral isosorbide dinitrate (20 to 60 mg, three times a day) and a beta-adrenergic blocker (propranolol, 20 to 60 mg, three times a day or metoprolol, 50 to 100 mg, twice a day). Ten patients were taking oral nifedipine (10 to 20 mg, three times a day).

Group III comprised 10 patients who underwent coronary angiography to exclude coronary artery disease, concomitant valve disease or cardiomyopathy. None had severe heart failure or thromboembolic episodes, conditions frequently associated with intravascular platelet activation (26,27). All had normal coronary arteries. Seven of the 10 patients were taking beta-blocker (20 to 60 mg propranolol, three times a day or metoprolol, 50 to 100 mg, twice a day), all patients were receiving oral isosorbide dinitrate (20 to 60 mg, three times a day) and 5 patients were taking oral nifedipine (10 to 20 mg, three times a day).

Group IV comprised eight patients with acute myocardial infarction diagnosed by electrocardiographic criteria and serial enzymatic measurements of plasma creatine kinase and creatine kinase, MB fraction. All patients were hospitalized within 12 hours after the onset of typical chest pain, which was not relieved by sublingual nitroglycerin. Three patients were studied on day 3 and five patients on day 2 after the onset of symptoms. At the time of blood withdrawal they had been receiving an intravenous medication consisting of porcine mucosal heparin (25,000 IU/24 h) and glycerol trinitrate (2 to 5 mg/h) for at least 24 hours. Six patients received additional nifedipine (10 to 20 mg, three times a day). None of the patients with myocardial infarction was taking a beta-blocker at the time of sample collection; beta-blockers were discontinued in two of these patients 48 hours before the investigation.

Sample collection and platelet preparation. All subjects gave their informed consent. After overnight fasting and cessation of oral medication, 200 ml of venous blood was obtained between 8 and 9 AM by clean venipuncture (19 gauge needle) and anticoagulated with 3.8% trisodium citrate (1 + 9, vol/vol).

Platelet-rich plasma was prepared by low speed centrifugation (180*g*, 15 minutes) and the platelet count was adjusted to 3×10^{11} platelets/liter with autologous platelet-poor plasma. For prostacyclin binding studies, platelets were washed once in 0.2 *M* Tris-maleate, pH 5.9, and resuspended in phosphate-buffered saline solution (0.9% sodium chloride and 0.154 *M* phosphate buffer, pH 7.4) to obtain a platelet count of 1×10^{12}/liter.

Materials. The following were obtained from NEN (Dreieich, West Germany): 9-^{3}H-PGI_2-methylester, 2,8-^{3}H-adenine, 8-^{14}C-adenosine-3′,5′-cyclic phosphate and fructose-^{14}C(U)-sucrose. Adenosine-5-diphosphate and unlabeled prostaglandins were purchased from Sigma Biochemicals (Munich, West Germany) and adenosine-3′,5′-monophosphate sodium salt was from PL-Biochemicals (Freiburg, West Germany). Prostaglandin I_2 (PGI_2) sodium salt was dissolved in 50 m*M* Tris-hydrochloric acid, pH 8.5, and kept frozen at −80°C. For binding experiments, 9-^{3}H-PGI_2-methylester was hydrolyzed to prostacyclin sodium salt as described by Hsi et al. (28). Tritium-labeled and unlabeled ZK 36374 were a generous gift from Dr. E. Schillinger (Schering AG, Berlin, West Germany). AG50W-X4 resin was obtained from Bio-Rad Laboratories (Munich, West Germany). All other reagents were of analytic grade and were obtained from Merck (Darmstadt, West Germany).

Radioactivity was counted in 5 ml Instagel (Packard) in a Tri-Carb scintillation counter (Packard). Double label

counting efficiencies were 52% for tritium and 80% for carbon-14.

Platelet prostacyclin receptor assay. Platelet prostacyclin binding was determined using tritiated prostacyclin sodium salt (specific activity 10 Ci/mmol). Aliquots of washed platelets were incubated with 3 n*M* 9-^{3}H-prostacyclin (about 100,000 disintegrations/min) and appropriate concentrations of unlabeled prostacyclin up to 20 μM. With this range of ligand concentrations, complete saturation of the binding sites was accomplished (Fig. 1). The extracellular space was monitored with ^{14}C-sucrose as described by Siegl et al. (19). Incubations were carried out in a final volume of 1 ml at 20°C for 5 minutes and terminated by centrifugation (14,000*g*, 20 seconds). Radioactivity in pellets and in supernatants was determined by liquid scintillation counting. Bound 9-^{3}H-prostacyclin was calculated after correction for spillover, background and extracellular space. Nonspecific binding, determined in the presence of 20 μM unlabeled prostacyclin, was 20 to 50% of the total bound radioactivity. In all experiments the inflection point of the binding saturation curve was reached (29). To determine the reproducibility of the method, platelet prostacyclin receptors of three healthy individuals were reinvestigated after 3 to 12 months. The interassay variance of the binding capacity (B_{max}) was 12 ± 3%.

Calculation of receptor data. Binding capacity and equilibrium dissociation constants were calculated by computerized analysis of the competition curves (NONLIN) using one or two binding site models based on the law of mass action. The analysis of the competition curves, assuming a two site model, resulted in identical values of equilibrium dissociation constants ($K_{D1} = K_{D2}$). Furthermore, the two site model did not improve the "goodness of fit" when saturation analysis was performed (30). Thus, we decided to interpret the experimental data assuming a single binding site and nonspecific binding according to the formula:

$$B = B_{max} \times L/(K_D + L) + C \times L,$$

where K_D = equilibrium dissociation constant; L = total ligand concentration; B_{max} = binding capacity; B = total bound ligand; and C × L = nonspecific binding.

Characterization of platelet prostacyclin binding. The equilibrium dissociation constant of 9-^{3}H-prostacyclin binding (K_D = 52 n*M*) was found to be in close agreement with the dose of prostacyclin required for half-maximal stimulation of cyclic AMP accumulation in ^{3}H-adenine prelabeled platelets (53 ± 6 n*M*, n = 3) (Fig. 1). To verify whether platelet prostacyclin binding was specific, the displacement of 3 n*M* 9-^{3}H-PGI_2 by prostaglandin E_1 (PGE_1), 6-oxo-PGE_1, 6-oxo-$PGF_{1\alpha}$ and PGD_2 was measured: PGE_1 and 6-oxo-PGE_1 share the platelet prostacyclin receptor, but their affinity is much lower as compared with PGI_2 (500 and 1,600 n*M*, respectively). PGD_2 and 6-oxo-$PGF_{1\alpha}$ exhibited no competition with platelet prostacyclin binding in concentrations up to 10 μM (Fig. 2).

The kinetics of platelet prostacyclin binding were investigated with the stable prostacyclin analog ZK 36374. The association rate of 10 n*M* ^{3}H-ZK 36374 was measured at 20°C. The observed rate constant (K_{obs}), as calculated from the slope of pseudo-first order kinetics, was 8.5×10^{-3} s^{-1}. The rate constant of dissociation (K_{-1}) of the ligand-receptor complex was determined by measurements of the time course of ^{3}H-ZK 36374 displacement after addition of unlabeled ZK 36374 (20 μM final concentration). A half-life of 140 seconds was found, from which $K_{-1} = 4.9 \times 10^{-3}$ s^{-1} could be determined. The forward rate constant is given by $K_{+1} = (K_{obs} - K_{-1})/(\text{ligand})$ and was 3.6×10^5 mol^{-1} s^{-1}. Thus, the true dissociation constant of ZK 36374 binding, which is given by the ratio K_{-1}/K_{+1}, could

Figure 1. Representative prostacyclin binding saturation curve (●) and dose-response curve of prostacyclin (PG)I_2-induced cyclic adenosine monophosphate (cAMP) accumulation (□) in intact washed platelets resuspended in phosphate-buffered saline solution. incorp. = incorporated.

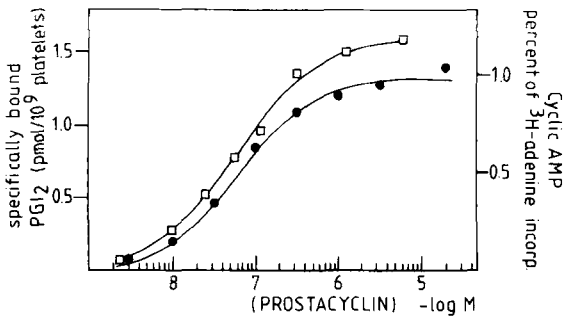
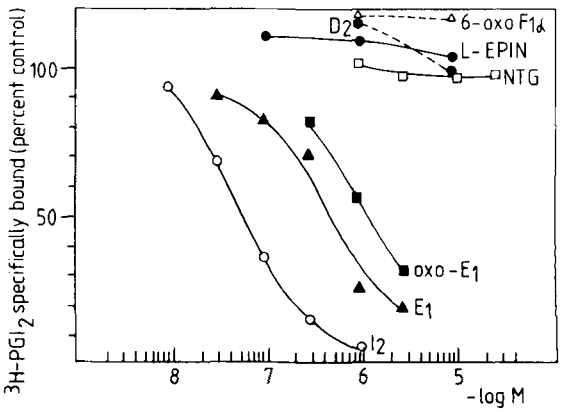


Figure 2. Displacement of specific binding of 9-^{3}H-prostacyclin (9-^{3}H-PGI_2) by prostaglandins, nitroglycerin and L-epinephrine. Platelets were incubated with 3 n*M* 9-^{3}H-PGI_2 and increasing amounts of unlabeled PGI_2 (I_2), PGE_1 (E_1), 6-oxo-PGE_1 (oxo-E_1), PGD_2 (D_2), 6-oxo-PGF_1 ($F_{1\alpha}$), nitroglycerin (NTG) and L-epinephrine (L-EPIN). Specific binding of labeled PGI_2 was determined in the presence of 20 μM PGI_2 and was 65 fmol/10^9 platelets. (**Data points** represent means of at least three determinations.)



be calculated to be 14 n*M*. This is in agreement with the equilibrium dissociation constant of ZK 36374 binding, which amounted to 10 to 15 n*M*, indicating that this prostacyclin analog has a two to three times higher affinity to prostacyclin binding sites than prostacyclin.

To investigate whether platelet prostacyclin receptors are regulated by their agonists, citrated platelet-rich plasma was incubated for 12 hours at 20°C with ZK 36374 in a final concentration of 100 n*M*. The incubation was terminated by thorough washing to remove the agonist. Complete removal was checked by adding ^{3}H-ZK 36374. Platelets preincubated with 100 n*M* ZK 36374 bound less of the stable prostacyclin analog compared with control platelets obtained from the same donors (−42 ± 7%, n = 3). This desensitization process has been demonstrated to be time- and dose-dependent ($t_{1/2}$ ~ 60 minutes, ED_{50} ~ 50 n*M*).

Cyclic AMP accumulation in platelets. Platelet-rich plasma was prelabeled with 0.6 μ*M* ^{3}H-adenine (specific activity 25 Ci/mmol). Incubations of prelabeled platelet-rich plasma with various concentrations of PGI_2 were carried out at 37°C for 10 minutes and were terminated by the addition of 50% (wt/vol) trichloroacetic acid containing appropriate amounts of ^{14}C-cyclic AMP to monitor recovery during the following purification steps. ^{3}H-cyclic AMP was isolated by chromatography on AG50W-X4 (H^+ form) and subsequently treated with zinc sulfate and barium hydroxide. The identity of the isolated ^{3}H-cyclic AMP was demonstrated by phosphodiesterase hydrolysis, and the amount formed expressed as percent of total radioactivity incorporated into the platelets.

Inhibitory effect of prostacyclin on adenosine diphosphate-induced aggregation. Platelet-rich plasma (3×10^{11} platelets/liter) was stirred in an aggregometer (Fresenius, Homburg, West Germany) with various amounts of prostacyclin for 90 seconds at 37°C before adenosine diphosphate was added to give a final concentration of 0.6 μ*M*. The primary aggregation induced by adenosine diphosphate was recorded and the dose of prostacyclin required for half-maximal inhibition (IC_{50}) was calculated after logit-log transformation.

Statistics. All results are expressed as means ± 1 SD. Statistical analysis was performed by nonparametric statistics (Mann-Whitney test); probability (p) values less than 0.01 were considered to be significant.

Results

Platelet prostacyclin binding and the inhibitory effect of prostacyclin on adenosine diphosphate-induced aggregation in vitro. The data of binding capacity (B_{max}) and affinity (K_D) of platelet prostacyclin binding sites, the antiaggregatory effects of prostacyclin (IC_{50}) and the extent and localization of coronary heart disease determined by angiography from patients with stable angina are listed in Table 1. Prostacyclin binding data, IC_{50} and diagnosis of patients with normal angiograms are shown in Table 2.

Table 1. Platelet Prostacyclin Binding, Antiaggregatory Potency of Prostaglandin I_2 and Angiographic Findings in 20 Patients With Stable Angina

Case	Age (yr)	IC_{50} (n*M*)	B_{max} (pmol/10^9plts)	K_D (n*M*)	Extent* and Localization of CAD	Previous Infarction† (mo)	GS
1	50	1.63	1.67	48	2 (RCA,LAD)	—	36
2	45	2.41	1.51	62	3 (RCA,LAD,LCx)	4	35
3	56	1.18	1.10	84	2 (LAD,LC)	6	31
4	54	2.53	2.97	111	2 (RCA,LCx)	—	4
5	48	1.51	1.16	44	2 (RCA,LAD)	—	12
6	47	2.48	0.63	41	1 (Diag)	—	4
7	53	2.02	1.67	33	2 (RCA,LCx)	12	4
8	53	0.81	1.29	67	2 (RCA,LAD)	22	52
9	51	0.85	1.90	104	3 (RCA,LAD,LCx)	3	59
10	54	1.32	2.14	144	3 (RCA,LAD,LCx)	—	28
11	49	1.22	2.15	114	2 (RCA,LCx)	1	34
12	59	1.13	1.21	77	3 (RCA,LAD,LCx)	—	39
13	52	1.54	1.06	70	1 (LAD)	—	10
14	60	1.06	1.18	68	2 (RCA,LAD)	—	19
15	61	1.65	2.03	90	3 (RCA,LAD,LCx)	7	35
16	54	1.05	1.24	59	2 (RCA,LAD)	4	60
17	53	1.08	1.74	68	3 (RCA,LAD,LCx)	1	41
18	57	1.39	1.41	88	2 (LAD,RCA)	1	49
19	55	0.82	1.10	54	3 (RCA,LAD,LCx)	—	32
20	51	0.92	0.74	26	1 (LAD)	—	3

*Number of vessels involved with stenosis greater than 50%. †Duration after myocardial infarction. B_{max} and K_D = binding capacity and affinity, respectively, of platelet prostacyclin receptors; CAD = coronary artery disease; Diag = ramus diagonalis; GS = Gensini coronary artery score; IC_{50} = dose of prostacyclin required for half-maximal inhibition of platelet aggregation; LAD = left anterior descending coronary artery; LC = left main coronary artery; LCx = left circumflex coronary artery; plts = platelets; RCA = right coronary artery; = no previous infarction.

Table 2. Platelet Prostacyclin Binding and Diagnosis of 10 Patients With Normal Coronary Angiograms

Case	Age (yr)	IC_{50} (n*M*)	B_{max} (pmol/10^9plts)	K_D (n*M*)	Diagnosis
1	60	1.62	1.76	104	Normal
2	51	1.18	1.67	48	Normal
3	55	1.26	0.92	64	MVD
4	36	1.23	1.28	73	CM
5	55	0.80	1.86	56	CM
6	53	1.02	1.12	57	MVD
7	41	0.92	0.74	26	Normal
8	45	1.16	2.24	110	MVD
9	63	1.47	1.86	71	CM
10	44	1.07	1.51	52	AVD

AVD = aortic valve disease; CM = cardiomyopathy; MVD = mitral valve disease; other abbreviations as in Table 1.

Patients with stable angina were not different from the age-matched control subjects with normal angiograms and the young healthy volunteers with respect to binding capacity, but binding affinity in these patients was lower than that found in young healthy volunteers. Nevertheless, no statistically significant difference was observed when comparing patients with stable angina with age-matched control subjects with normal angiograms. The inhibitory effect of prostacyclin on platelet aggregation induced by adenosine diphosphate also did not differ from that in control subjects (Table 3). There is obviously no correlation between the extent and severity of coronary artery disease and platelet prostacyclin binding data or in vitro responsiveness to prostacyclin (Table 2).

In patients with acute myocardial infarction, however, platelet prostacyclin binding capacity was significantly reduced (by 49%, $p = 0.001$), but binding affinity did not differ from that in control subjects. In the two patients in whom beta-blockade was stopped 2 days before the investigation, the binding capacity of platelet prostacyclin receptors was 1.17 and 0.24 pmol/10^9 platelets, respectively. The reduction of platelet prostacyclin receptor density in acute myocardial infarction was demonstrated to be reversible. Two patients were restudied at day 26 and day 15, respectively, after myocardial infarction: the binding capacity of both had returned to normal levels (1.80 and 1.41 pmol/10^9 platelets).

In patients with acute myocardial infarction, the inhibitory effect of prostacyclin on platelet aggregation induced by adenosine diphosphate was also reduced (IC_{50} increased by 67%). Primary platelet aggregation, determined by change of optical density after addition of 0.6 μM adenosine diphosphate, was not enhanced in this group (Table 3).

To rule out direct prostacyclin binding impairment due to heparin, which was given intravenously to all patients with myocardial infarction, two types of in vitro experiments were carried out: 1) Washed platelets from healthy volunteers were incubated with 3 n*M* labeled prostacyclin and increasing amounts of porcine mucosal heparin (3 to 250 IU/ml). No displacement of specifically bound prostacyclin by heparin could be demonstrated. 2) Citrated platelet-rich plasma was incubated with 4 IU/ml porcine mucosal heparin, a concentration clearly above expected plasma levels after 25,000 IU/24 hours of intravenous heparin. After 30 minutes of incubation at 30°C, platelets were washed and the prostacyclin binding capacity was determined. No differences were observed in binding capacity and affinity be-

Table 3. Platelet Prostacyclin Binding, Antiaggregatory Potency of Prostaglandin I_2, Platelet Aggregation Induced by Adenosine Diphosphate (ADP) and Plasma Lipids in the Four Subgroups of Subjects

	Group			
	I Healthy Volunteers (n = 14)	II Normal Angiograms (n = 10)	III Stable Angina (n = 20)	IV Myocardial Infarction (n = 8)
B_{max} (pmol/10^9plts)	1.35 ± 0.37	1.50 ± 0.47	1.51 ± 0.54 (0.300/0.421)	0.69 ± 0.45 (0.001/0.003)
K_D (n*M*)	52 ± 13	66 ± 25	74 ± 28 (0.006/0.214)	42 ± 25 (0.123/0.038)
IC_{50} (n*M*)	1.12 ± 0.22	1.17 ± 0.24	1.43 ± 0.54 (0.049/0.136)	1.87 ± 0.59 (0.005/0.003)
ADP (% LT)	15.2 ± 3.8	13.7 ± 4.6	15.2 ± 4.4 (0.500/0.079)	14.2 ± 3.6 (0.263/0.313)
Cholesterol (m*M*)	4.12 ± 0.67	4.89 ± 1.0	4.89 ± 0.62 (0.003/0.372)	4.32 ± 0.90 (0.471/0.084)
Triglycerides (m*M*)	1.49 ± 0.63	1.16 ± 0.67	1.67 ± 0.67 (0.039/0.022)	1.30 ± 0.76 (0.457/0.395)
Age (yr)	31 ± 3	54 ± 8	55 ± 6	56 ± 8
Sex (M/F)	14/0	10/0	20/0	7/1

Data are given as mean ± SD; probability values are given in parentheses (III to IV versus I/III to IV versus II). F = female; LT = light transmission; M = male; other abbreviations as in Table 1.

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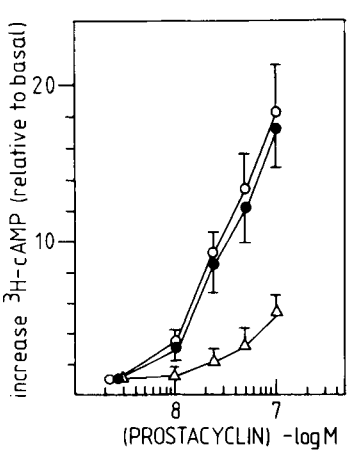
tween heparin-incubated platelets and control platelets obtained from the same donor.

Further competition experiments showed no evidence that nitroglycerin in concentrations ranging from 0.8 to 22 μM interfered with specific prostacyclin binding. Also, L-epinephrine did not displace specifically bound prostacyclin in concentrations of up to 10 μM (Fig. 2).

Prostacyclin-induced cyclic AMP accumulation in ^{3}H-adenine prelabeled platelets. In intact platelets, more than 90% of cyclic AMP synthesized after stimulation of platelet adenylate cyclase is hydrolyzed by phosphodiesterases. This regulation of cyclic AMP levels is not apparent when adenylate cyclase activity in platelet membranes is being measured. Because the determination of the amount of immunoreactive cyclic AMP in platelet-rich plasma is compromised by the high background levels of plasma cyclic AMP, prostacyclin-induced platelet cyclic AMP synthesis was measured using the ^{3}H-adenine prelabeling technique.

Six healthy volunteers (group I), nine patients with stable angina (group II) and four patients with acute myocardial infarction (group III) were investigated. The dose of prostacyclin required for doubling the cyclic AMP content of platelets (EC_{200}) was similar in groups I and II (5.2 ± 0.4 versus 5.3 ± 1.7 nM). However, the four patients with acute myocardial ischemia required considerably more prostacyclin for doubling the cyclic AMP of their platelets (22.0 ± 4.5 nM). The increase of cyclic AMP did not differ in groups I and II (17.3 ± 2.1 versus 18.3 ± 2.6 times greater increase in the presence of 100 nM prostacyclin) (Fig. 3). In the patients with acute myocardial infarction, however, prostacyclin caused a less pronounced increase in intracellular cyclic AMP (5.5 ± 1.0 times greater).

Figure 3. Prostaglandin I_2-induced cyclic adenosine monophosphate (cAMP) accumulation in platelet-rich plasma prelabeled with ^{3}H-adenine in healthy volunteers (●), patients with stable angina (○) and acute myocardial infarction (△). Data are given as increase of intracellular cyclic AMP content relative to basal levels, which were not different among the groups and were less than 0.1% of total radioactivity in the platelets. Symbols represent means ± SD.



To rule out the possibility that heparin interferes with the prostacyclin-induced cyclic AMP synthesis in intact platelets, we showed that the addition of 60 IU/ml heparin had no influence on cyclase stimulation by 10 nM prostacyclin.

Discussion

In preliminary experiments, binding of prostacyclin to intact platelets was found to be linearly correlated with platelet number and was saturable, reversible and specific. Thus, it met all criteria for a specific binding site. The equilibrium dissociation constant of prostacyclin binding determined by direct binding studies was in close agreement with the dose of prostacyclin required for half-maximal stimulation of cyclic AMP synthesis in platelets. The evaluation of prostacyclin binding data by nonlinear fitting, using binding models based on the law of mass action, did not show a significantly better fit by a two binding site model. Therefore, in contrast to previous reports (19,20), binding data were evaluated assuming a single binding site and nonspecific binding (30).

Stable angina. In patients with stable angina and angiographically documented coronary artery disease, no alteration of binding capacity and affinity for platelet prostacyclin receptors was found. The prostacyclin-induced cyclic AMP accumulation in intact platelets, which reflects adenylate cyclase as well as phosphodiesterase activity, was comparable in patients with stable angina and in young healthy control subjects. In contrast to other investigators (17,18), we did not find a significant difference in platelet responsiveness to prostacyclin. This might be due to differences in methods and patient selection (2). We determined the inhibitory effect of prostacyclin on primary platelet aggregation induced by adenosine diphosphate, because secondary aggregation elicited by adenosine diphosphate depends on the synthesis of cyclic endoperoxides and thromboxanes (31) and synthesis of thromboxane B_2 has been demonstrated to be increased in patients with ischemic heart disease (14,15). Furthermore, secondary aggregation seems to be of questionable physiologic relevance because it occurs only in a calcium-depleted medium, such as citrated platelet-rich plasma (32). It also must be emphasized that patients with stable angina and control patients with negative angiographic findings were comparable with respect to their plasma lipids, age, medication and sample collection, factors that might modify the sensitivity of platelets to prostacyclin (33,34).

Acute myocardial infarction. Patients with acute myocardial ischemia exhibited a considerably lower platelet responsiveness to prostacyclin and prostacyclin-induced cyclic AMP accumulation. Because heparin was given to all these patients, its effects on platelet phosphatidylinositol metabolism and prostaglandin-stimulated adenylate cyclase activity could account for these observations (35,36). However, there was no evidence that heparin interfered with prosta-

cyclin-induced cyclic AMP accumulation when intact platelets were studied instead of platelet membranes. Because adenylate cyclase is presumed to be localized at the inner surface of the platelet membrane, a possible explanation could be that highly charged molecules like heparin probably have limited, if any, access to the enzyme in intact cells. Although heparin did not interfere with platelet prostacyclin binding as determined in vitro by competition experiments, reduced binding capacity of the binding sites was found in patients with myocardial infarction. In addition, competition experiments showed no displacement of specifically bound prostacyclin by nitroglycerin or L-epinephrine. Thus, the reduced antiaggregatory effectiveness and the impaired cyclic AMP accumulation after addition of prostacyclin most likely reflect impaired receptor response due to reduction of prostacyclin receptor density.

The reason for the reduced binding capacity of platelet prostacyclin receptors in acute myocardial infarction remains unknown. The increased vascular prostacyclin biosynthesis in patients with severe atherosclerosis and the concomitant platelet activation stimulated the speculation that agonist-induced prostacyclin receptor regulation might occur in vivo, because reduced platelet responsiveness to prostacyclin has been observed in these patients (37,38). If indeed there is an agonist-induced receptor regulation, it is conceivable that platelet prostacyclin receptor density is unchanged in patients with stable angina because urinary excretion of dinor-prostacyclin metabolites is not altered in this group (37). In patients with acute myocardial infarction, increased urinary excretion of 6-oxo-$PGF_{1\alpha}$, the stable hydrolysis product of prostacyclin, was reported (39). However, urinary excretion of this compound reflects complex renal prostaglandin synthesis and excretion and is not a reliable estimation of systemic vascular prostacyclin formation (40,41). The influence of intravenous nitroglycerin, which was given to all patients with acute myocardial infarction, on vascular prostacyclin synthesis is controversial (42–44). Nevertheless, because intravascular platelet activation is a frequent finding during myocardial infarction as shown by beta-thromboglobulin determinations, shortened platelet survival and increased urinary excretion of 2,3-dinor-thromboxane B_2 (45–47), one might expect that prostacyclin biosynthesis is increased (37). Recently, this concept has been substantiated by the determination of urinary excretion of 2,3-dinor-6-oxo-$PGF_{1\alpha}$ in patients with acute myocardial infarction and unstable angina (48). Furthermore, as shown by in vitro experiments (see Methods), there is indeed some evidence that platelet prostacyclin receptors are regulated by agonist-induced receptor changes: incubation of platelet-rich plasma with the stable prostacyclin analog ZK 36374 resulted in a time- and dose-dependent loss of receptors without significant changes in binding affinity. Thus it seems unlikely that prostacyclin causes upregulation of its own binding sites, as postulated by Neri Serneri et al. (49).

Conclusions. Our data provide no evidence that in patients with stable angina a loss of prostacyclin receptors might be a further pathogenetic factor predisposing to thrombotic occlusion or vasospasm. The impairment of platelet prostacyclin binding in patients with myocardial infarction could favor the accumulation of platelets in the poststenotic segment of the coronary artery. It has been suggested that these platelet aggregates are responsible for cyclic flow reduction as a result of release of thromboxane A_2 (50). In acute myocardial infarction, application of prostacyclin or its stable analogs has been considered a promising therapeutic approach (51,52). Long-term application of prostacyclin, however, is followed by desensitization of platelets against the antiaggregatory effects of prostacyclin and prostacyclin-induced cyclic AMP accumulation (53,54). Because platelet prostacyclin binding is already reduced in acute myocardial infarction, one might expect an enhanced rebound effect, resulting in hyperaggregability of platelets after cessation of prostacyclin therapy, with potential reocclusion and infarct extension. Thus, rebound effects should be avoided by slowly tapering off prostacyclin infusion; overlapping treatment with platelet inhibitors may be an alternative approach.

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